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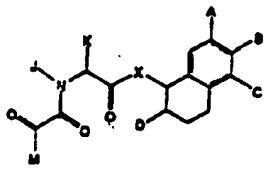
(54) Title: TETRALIN COMPOUNDS WITH MDR ACTIVITY

(57) Abstract

The present invention relates to compounds that can maintain, increase, or restore sensitivity of cells to therapeutic or prophylactic agents. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for treatment of multi-drug resistant cells, for prevention of the development of multi-drug resistance, and for the use in multi-drug resistant cancer. These compounds are represented by general formula (I), in which the different substitutes are defined into the description.



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TETRALIN COMPOUNDS WITH MDR ACTIVITY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to novel compounds which can maintain, increase, or restore sensitivity of cells to therapeutic or prophylactic agents. This invention also relates to pharmaceutical compositions and methods utilizing these compounds. The methods of this invention are directed to the treatment of multi-drug resistant cells, preventing the development of multi-drug resistance and use in multi-drug resistant cancer therapy.

BACKGROUND OF THE INVENTION

A major problem affecting the efficacy of chemotherapy regimens is the evolution of cells which, upon exposure to a chemotherapeutic drug, become resistant to a multitude of structurally unrelated drugs and therapeutic agents. The appearance of such multi-drug resistance often occurs in the presence of over-expression of a 170-kDA membrane P-glycoprotein (gp-170). The gp-170 protein is present in the plasma membranes of some healthy tissues, in addition to cancer cell lines, and is homologous to bacterial transport proteins (Hait et al., Cancer Communications, 1(1), p. 35 (1989); West, TIBS, 15, p. 42 (1990)). The protein acts as an export pump, conferring drug resistance through active extrusion of toxic chemicals. Although the mechanism for the pump

is unknown, it is speculated that the gp-170 protein functions by expelling substances that share certain chemical or physical characteristics, such as hydrophobicity, the presence of carbonyl groups, or the existence of a glutathione conjugate (see West).

Recently, another protein responsible for multi-drug resistance, MRP (multi-drug resistance associated protein), was identified in H69AR cells, an MDR cell line that lacks detectable P-glycoprotein [S. P. C. Cole et al., Science, 258, pp. 1650-54 (1992)]. MRP has also been detected in other non-P-glycoprotein MDR cell lines, such as HL60/ADR and MCF-7 breast carcinoma cells [(E. Schneider et al., Cancer Res., 54, pp. 152-58 (1994); and N. Krishnamachary et al., Cancer Res., 53, pp. 3658-61 (1993)].

The MRP gene encodes a 190 kD membrane-associated protein that is another member of the ATP binding cassette superfamily. MRP appears to function in the same manner as P-glycoprotein, acting as a pump for removing natural product drugs from the cell. A possible physiological function for MRP maybe ATP-dependent transport of glutathione S-conjugates [G. Jedlitschky et al., Cancer Res., 54, pp. 4833-36 (1994); I. Leier et al., J. Biol. Chem., 269, pp. 27807-10 (1994); and Muller et al., Proc. Natl. Acad. Sci. USA, 91, pp. 13033-37 (1994)].

The role of MRP in clinical drug resistance remains to be clearly defined, but it appears likely that MRP may be another protein responsible for a broad resistance to anti-cancer drugs.

Various chemical agents have been administered to repress multi-drug resistance and restore drug sensitivity. While some drugs have improved the responsiveness of multi-drug resistant ("MDR") cells to chemotherapeutic agents, they have often been accompanied

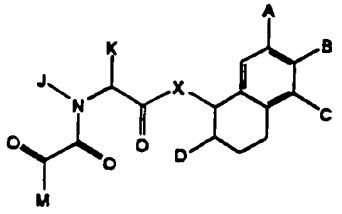
preventing and reversing multi-drug resistant ("MDR"). The compounds of this invention may be formulated into pharmaceutical compositions useful to maintain the therapeutic or prophylactic effects of drugs in cells, or to restore those effects in MDR cells. Such compositions may optionally contain additional therapeutic or prophylactic agents.

According to another embodiment, the invention provides methods of utilizing the above pharmaceutical compositions for treating or preventing both P-glycoprotein- and MRP-mediated MDR. Such methods are especially useful to enhance the efficacy of chemotherapy regimens employed in the treatment of cancer or other diseases.

The present invention also provides methods for preparing the compounds of this invention.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a novel class of compounds represented by formula (I):



Formula (I)

and pharmaceutically acceptable salts thereof, wherein:

A, B and C are independently selected from hydrogen, halogen, (C1-C6)-straight or branched alkyl, O-(C1-C6)-straight or branched alkyl, $(CH_2)_n$-Ar or $Y(CH_2)_n$-Ar; wherein

Y is O, S or NR_1; wherein

R_1 is (C1-C6)-straight or branched alkyl and hydrogen;

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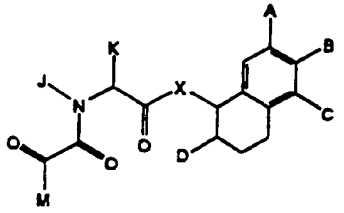

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Y is O, S or NR_1; wherein

R_1 is (C1-C6)-straight or branched alkyl and hydrogen;

E is Ar or NR_4R_5; wherein

R_4 and R_5 are independently selected from hydrogen, (C1-C5)-straight or branched alkyl and (CH_2)Ar or can be taken together to form a 5 or 6 membered heterocyclic ring; and

m is an integer from 1 to 3;

X is O or NR_6; wherein

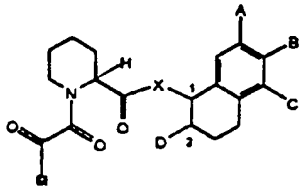
R_6 is selected from the group consisting of hydrogen, (C1-C6)-straight or branched alkyl and $(CH_2)_m$-Ar;

J and K are independently (C1-C6)-straight or branched alkyl or Ar-substituted with (C1-C6)-straight or branched alkyl or wherein J and K are taken together to form a five or six membered ring or a five or six membered benzo-fused ring;

M is (C1-C6)-straight or branched alkyl or Ar; and

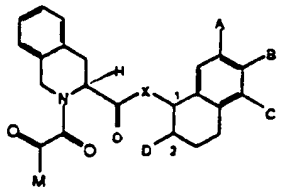
the stereochemistry at carbon 1 and carbon 2 is independently selected from R or S.

More preferred compounds of this invention are represented by formula (II):



Formula (II) ;

formula (III):



Formula (III) ;

and formula (IV):

E is Ar or NR_4R_5; wherein

R_4 and R_5 are independently selected from hydrogen, (C1-C5)-straight or branched alkyl and $(CH_2)Ar$ or can be taken together to form a 5 or 6 membered heterocyclic ring; and

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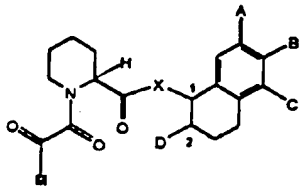

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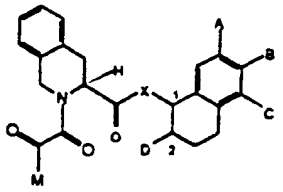

Formula (III) ;

and formula (IV):

Cpd	Formula	A	B	C	D	J	K	X
30A	II	O-propyl	methyl	O-propyl	(CH_2)-3-Pyr			O
30B	II	O-propyl	methyl	O-propyl	(CH_2)-3-Pyr			O

As defined herein, the compounds of this invention include all optical and racemic isomers.

In addition to the compounds described herein, the invention also includes pharmaceutically acceptable derivatives of those compounds. A "pharmaceutically acceptable derivative" denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of this invention or any other compound which, upon administration to a patient, is capable of providing (directly or indirectly) a compound of this invention, or a metabolite or residue thereof, characterized by the ability to maintain, increase or restore sensitivity of MDR cells to therapeutic or prophylactic agents or to prevent development of multi-drug resistance.

Compounds of this invention represented by formula (I) may be obtained using any conventional technique. Preferably, these compounds are chemically synthesized from readily available starting materials, such as alpha-amino acids. Modular and convergent methods for the synthesis of these compounds are also preferred. In a convergent approach, for example, large sections of the final product are brought together in the last stages of the synthesis, rather than by incremental addition of small pieces to a growing molecular chain.

Scheme 1 illustrates a representative example of a convergent process for the synthesis of compounds of formula (I). The process comprises coupling of a protected amino acid of formula (VI), wherein P is a protecting group, with an amine or alcohol of formula (V), wherein X is O or NR_6 to provide an ester (when X = O) or an amide (when X = NR_6) of formula (VII). Protected

Cpd	Formula	A	B	C	D	J	K	X
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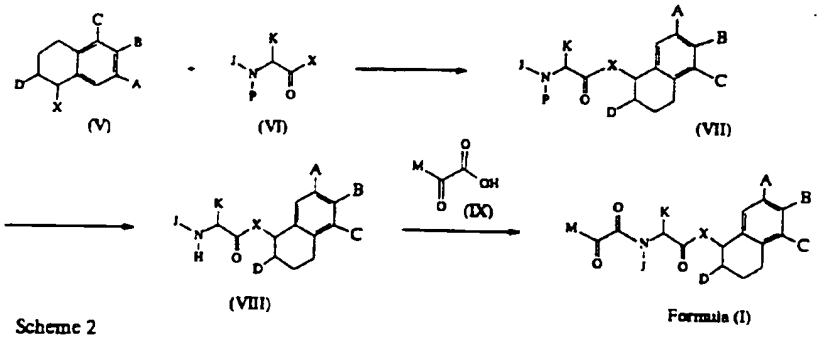
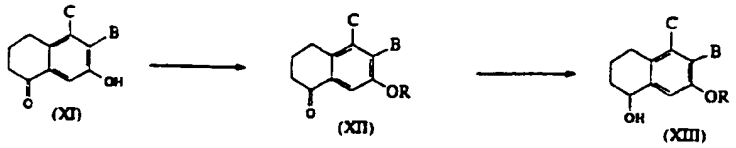
Scheme 1

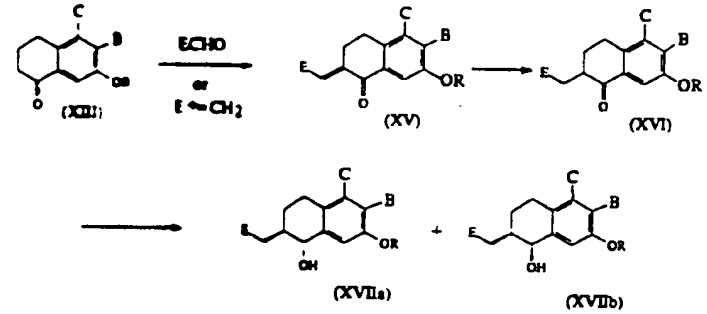


Scheme 2



Scheme 3

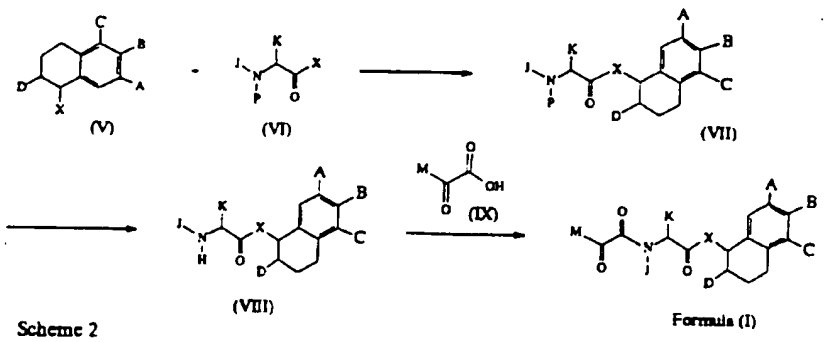
Scheme 4



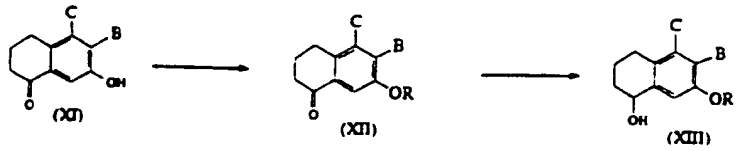
Thus, this invention also provides a method for preparing compounds of formula (I) comprising the steps of:

(a) coupling an amino acid of formula (VI) with an alcohol or amine of formula (V), wherein X is O

Scheme 1

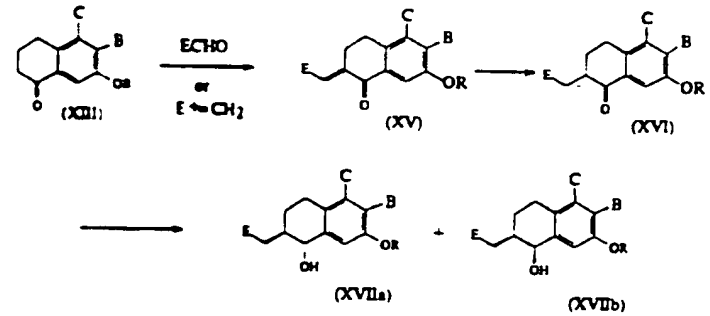


Scheme 2



Scheme 3

Scheme 4



Thus, this invention also provides a method for preparing compounds of formula (I) comprising the steps of:

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of this invention may be synthesized. Further methods or modifications of the above general schemes will be evident to those of ordinary skill in the art.

The compounds of this invention may be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

The compounds of this invention are characterized by the ability to increase, restore or maintain the sensitivity of MDR cells to cytotoxic compounds, such as, for example, those typically used in chemotherapy. Based on that ability, the compounds of this invention are advantageously used as chemosensitizing agents, to increase the effectiveness of chemotherapy in individuals who are afflicted with drug-resistant cancers, tumors, metastases or disease. In addition, the compounds of this invention are capable of maintaining sensitivity to therapeutic or prophylactic agents in non-resistant cells. Therefore, the compounds of this invention are useful in treating or preventing multi-drug resistance ("MDR") in a patient. More specifically, these compounds are useful in treating of preventing P-glycoprotein-mediated MDR and MRP-mediated MDR.

As used throughout this application, the term "patient" refers to mammals, including humans. And the term "cell" refers to mammalian cells, including human cells.

As used herein, the terms "sensitizing agent", "sensitizer", "chemosensitizing agent", "chemo-

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As used herein, the terms "sensitizing agent", "sensitizer", "chemosensitizing agent", "chemo-

such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions of this invention comprise any of the compounds of the present invention, or pharmaceutically acceptable salts thereof, with any pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

According to this invention, the pharmaceutical compositions may be in the form of a sterile injectable

such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

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According to this invention, the pharmaceutical compositions may be in the form of a sterile injectable

of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical

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For ophthalmic use, the pharmaceutical

When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this invention may comprise a combination of a compound of this invention and another therapeutic or prophylactic agent.

For example, the compounds may be administered either alone or in combination with one or more therapeutic agents, such as chemotherapeutic agents, (e.g., actinomycin D, doxorubicin, vincristine, vinblastine, etoposide, amsacrine, mitoxantrone, tenipaside, taxol and colchicine) and/or a chemosensitizing agent (e.g., cyclosporin A and analogs, phenothiazines and thioxantheres), in order to increase the susceptibility of the MDR cells within the patient to the agent or agents.

According to another embodiment, the invention provides methods for treating or preventing multi-drug resistance in a patient by administering a composition comprising an effective amount of a compound of this invention. Effective dosage levels for treating or preventing MDR range from between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 50 mg/kg body weight per day of a compound of this invention. A typical composition for use in treating MDR will contain between about 5% and about 95% of active compound(s) (w/w), whether it be solely one of the compounds of this invention or a combination of a compound of this invention and another chemotherapeutic or chemosensitizing agent. Preferably, such preparations contain between about 20% and about 80% active compound(s).

In order that this invention may be more fully understood, the following examples are set forth. These

When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this invention may comprise a combination of a compound of this invention and another therapeutic or prophylactic agent.

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According to another embodiment, the invention provides methods for treating or preventing multi-drug resistance in a patient by administering a composition comprising an effective amount of a compound of this invention. Effective dosage levels for treating or preventing MDR range from between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 50 mg/kg body weight per day of a compound of this invention. A typical composition for use in treating MDR will contain between about 5% and about 95% of active compound(s) (w/w), whether it be solely one of the compounds of this invention or a combination of a compound of this invention and another chemotherapeutic or chemosensitizing agent. Preferably, such preparations contain between about 20% and about 80% active compound(s).

In order that this invention may be more fully understood, the following examples are set forth. These

Example 2

7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-ol (Compound 2):

To a solution of Compound 1 (16.41 g, 64.9 mmol) in tetrahydrofuran (75 mL) at 0°C was added dropwise a 1M solution of diisobutylaluminum hydride in toluene (97.3 mL). After 1 hr, the reaction was quenched with aqueous potassium sodium tartrate and diluted with ethyl acetate followed by warming to room temperature. After stirring for an additional hour, the layers were separated and the aqueous phase was re-extracted with ethyl acetate (2x). The extracts were combined, washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with ethyl acetate) provided 12.96 g of Compound 2 as an oil which crystallized upon standing.

Example 3

7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-ol (Compound 2 (S)) and 1(R)-Accedas-7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 3(R)):

A solution of Compound 2 (12.96, 50.82 mmol) in tetrahydrofuran (20 mL) was diluted with tert-butylmethyl ether (260 mL) followed by the addition of vinyl acetate (19.1 mL, 0.21 mol) and Amano PS-30 Lipase (13.0 g). After stirring for 8 hrs, the reaction was filtered and concentrated in vacuo to provide an oil. Chromatography on silica gel (elution with 20% acetone:hexanes) provided 7.41 g of acetate 3(R) as a white crystalline material. Further elution with 60% acetone:hexanes provided 6.1 g of Compound 2(S) as a white crystalline material. The enantiomeric purity of compound 2(S) was established by

Example 2

7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-ol (Compound 2):

To a solution of Compound 1 (16.41 g, 64.9 mmol) in tetrahydrofuran (75 mL) at 0°C was added dropwise a 1M solution of diisobutylaluminum hydride in toluene (97.3 mL). After 1 hr, the reaction was quenched with aqueous potassium sodium tartrate and diluted with ethyl acetate followed by warming to room temperature. After stirring for an additional hour, the layers were separated and the aqueous phase was re-extracted with ethyl acetate (2x). The extracts were combined, washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with ethyl acetate) provided 12.96 g of Compound 2 as an oil which crystallized upon standing.

Example 3

7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-ol (Compound 2 (S)) and 1(R)-Accedas-7-(Pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 3(R)):

A solution of Compound 2 (12.96, 50.82 mmol) in tetrahydrofuran (20 mL) was diluted with tert-butylmethyl ether (260 mL) followed by the addition of vinyl acetate (19.1 mL, 0.21 mol) and Amano PS-30 Lipase (13.0 g). After stirring for 8 hrs, the reaction was filtered and concentrated in vacuo to provide an oil. Chromatography on silica gel (elution with 20% acetone:hexanes) provided 7.41 g of acetate 3(R) as a white crystalline material. Further elution with 60% acetone:hexanes provided 6.1 g of Compound 2(S) as a white crystalline material. The enantiomeric purity of compound 2(S) was established by

Chromatography of the residue on silica gel (elution with 20% acetone:hexanes) provided 940 mg of Compound 4 as a mixture of diastereomers.

Example 6

(S)-Piperidine-2-carboxylic acid 2-((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) ester (Compound 5):

To a solution of Compound 4 (940 mg, 2.09 mmol) in tetrahydrofuran (5.0 mL)was added morpholine (1.1 mL, 12.6 mmol) and tetrakistriphenylphosphine pallidium (0) (241 mg, 0.21 mmol). After 1 hr, the heterogenous mixture was diluted with ethyl acetate, washed with 50% brine, 5% sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 50-100% acetone:hexanes) provided 510 mg of Compound 5.

Example 7

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester (Compound 6) and 1-(2-Oxo-2(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((7-pyridin-4-yl methoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 7):

To a solution of Compound 5 (510 mg, 1.4 mmol) and 3,4,5-trimethoxybenzyolformic acid (505 mg, 2.1 mmol) in methylene chloride (6 mL) was added (3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (400 mg, 2.1 mmol). After stirring for 24 hr, the reaction was diluted with ethyl acetate and water. The layers were separated and the aqueous phase was re-extracted with ethyl acetate. The extracts were combined, washed with sat.

Chromatography of the residue on silica gel (elution with 20% acetone:hexanes) provided 940 mg of Compound 4 as a mixture of diastereomers.

Example 6

(S)-Piperidine-2-carboxylic acid 2-((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) ester (Compound 5):

To a solution of Compound 4 (940 mg, 2.09 mmol) in tetrahydrofuran (5.0 mL)was added morpholine (1.1 mL, 12.6 mmol) and tetrakistriphenylphosphine pallidium (0) (241 mg, 0.21 mmol). After 1 hr, the heterogenous mixture was diluted with ethyl acetate, washed with 50% brine, 5% sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 50-100% acetone:hexanes) provided 510 mg of Compound 5.

Example 7

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester (Compound 6) and 1-(2-Oxo-2(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((7-pyridin-4-yl methoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 7):

To a solution of Compound 5 (510 mg, 1.4 mmol) and 3,4,5-trimethoxybenzoylformic acid (505 mg, 2.1 mmol) in methylene chloride (6 mL) was added (3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (400 mg, 2.1 mmol). After stirring for 24 hr, the reaction was diluted with ethyl acetate and water. The layers were separated and the aqueous phase was re-extracted with ethyl acetate. The extracts were combined, washed with sat.

To a solution of Compound 13A (601 mg, 1.08 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (1 mL). After stirring for 1.5 hr, the reaction was concentrated in vacuo. The residue was neutalized with sat. potassium carbonate and extracted with ethyl acetate (2x). The extracts were combined washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo, to provide 450 mg of Compound 14.

Example 15

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-(N-benzyl (7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) amide (Compound 15):

Compound 15 was prepared according to Example 7, but replacing Compound 5 with 14. ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$)δ 8.52 (d), 8.39 (dd), 7.51 (m), 7.44 (s), 7.37 (s), 7.37 (t), 7.30-7.15 (m), 7.09 (d), 7.05 (d), 6.99 (d), 6.89 (dd), 6.74 (m), 6.39 (m), 5.69 (d), 5.41 (m), 5.21 (m), 5.15 (q), 4.90 (q), 4.72 (d), 4.64 (d), 3.95-3.86 (m), 3.70-3.67 (m), 3.57 (br d), 3.54 (d), 3.48 (m), 2.74-2.64 (m), 2.20-1.58 (m).

Example 16

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid (2-N-benzyl (7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) amide (Compound 16):

Compound 16 was prepared according to Example 14-15, but replacing Compound 13A with 13B. ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.63 (d), 7.37-7.33 (m), 7.30-7.22 (m), 7.13-7.10 (m), 7.03 (dd), 6.87 (br s), 6.79 (dt), 5.83 (m), 5.06 (q), 4.96 (q), 4.90 (d), 4.83 (q), 4.38 (d), 4.13 (d), 3.94 (s), 3.90 (s), 3.87 (s), 3.85 (s), 2.70-2.62 (m), 2.14 (m), 1.91 (m), 1.88-1.68 (m),

To a solution of Compound 13A (601 mg, 1.08 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (1 mL). After stirring for 1.5 hr, the reaction was concentrated in vacuo. The residue was neutalized with sat. potassium carbonate and extracted with ethyl acetate (2x). The extracts were combined washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo, to provide 450 mg of Compound 14.

Example 15

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-(N-benzyl (7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) amide (Compound 15):

Compound 15 was prepared according to Example 7, but replacing Compound 5 with 14. ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.52 (d), 8.39 (dd), 7.51 (m), 7.44 (s), 7.37 (s), 7.37 (t), 7.30-7.15 (m), 7.09 (d), 7.05 (d), 6.99 (d), 6.89 (dd), 6.74 (m), 6.39 (m), 5.69 (d), 5.41 (m), 5.21 (m), 5.15 (q), 4.90 (q), 4.72 (d), 4.64 (d), 3.95-3.86 (m), 3.70-3.67 (m), 3.57 (br d), 3.54 (d), 3.48 (m), 2.74-2.64 (m), 2.20-1.58 (m).

Example 16

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid (2-N-benzyl (7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) amide (Compound 16):

Compound 16 was prepared according to Example 14-15, but replacing Compound 13A with 13B. ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.63 (d), 7.37-7.33 (m), 7.30-7.22 (m), 7.13-7.10 (m), 7.03 (dd), 6.87 (br s), 6.79 (dt), 5.83 (m), 5.06 (q), 4.96 (q), 4.90 (d), 4.83 (q), 4.38 (d), 4.13 (d), 3.94 (s), 3.90 (s), 3.87 (s), 3.85 (s), 2.70-2.62 (m), 2.14 (m), 1.91 (m), 1.88-1.68 (m),

diastereomers. ^{1}H NMR as a mixture of diastereomers and rotomers (500 MHz, $CDCl_3$) δ 8.59 (d), 7.38 (s), 7.37 (s), 7.33 (m), 7.22 (d), 7.18 (dd), 7.04 (d), 6.77 (dt), 6.70 (m), 6.64 (m), 6.04 (m), 5.92 (t), 5.88 (t), 5.35 (m), 5.06 (s), 5.05 (s), 5.03 (s), 4.58 (m), 4.31 (dd), 3.94 (s), 3.93 (s), 3.92 (s), 3.87 (s), 3.86 (s), 3.47 (br d), 3.27 (dq), 3.13 (dt), 3.07 (dt), 2.87-2.61 (m), 2.34 (br d), 2.26 (br d), 2.18-1.18 (m).

Example 9

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((5-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) ester (Compound 9):

Compound 9 was prepared as described in Examples 1-2 and 5-7 utilizing 5-hydroxy-1-tetralone in place of 7-hydroxy-1-tetralone to provide Compound 9 as a mixture of diastereomers. ^{1}H NMR as a mixture of diastereomers and rotomers (500 MHz, $CDCl_3$) δ 8.64 (m), 7.39 (m), 7.27 (s), 7.20 (d), 7.17 (q), 6.98 (d), 6.92 (d), 6.80 (t), 6.73 (dd), 6.40 (d), 6.10 (q), 5.99 (t), 5.95 (t), 5.40 (m), 5.12 (m), 5.12 (s), 5.08 (d), 4.60 (m), 4.35 (m), 3.96 (s), 3.85 (s), 3.94 (s), 3.90 (s), 3.89 (s), 3.50 (br d), 3.30 (dq), 3.19-3.08 (m), 3.0-2.86 (m), 2.74-2.58 (m), 2.38 (m), 2.30 (m), 2.10-1.50 (m), 1.45-1.25 (m).

Example 10

1-Amino-7-(pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 10):

To a solution of Compound 1 (1.71 g, 6.75 mmol) and methoxyamine hydrochloride (845 mg, 10.12 mmol) in abs. ethanol (20 mL) was added powdered potassium carbonate (2.25 g, 16.88 mmol) and the reaction heated to reflux. After 2 hr, the reaction was cooled and concentrated in

diastereomers. ^{1}H NMR as a mixture of diastereomers and rotomers (500 MHz, $CDCl_3$) δ 8.59 (d), 7.38 (s), 7.37 (s), 7.33 (m), 7.22 (d), 7.18 (dd), 7.04 (d), 6.77 (dt), 6.70 (m), 6.64 (m), 6.04 (m), 5.92 (t), 5.88 (t), 5.35 (m), 5.06 (s), 5.05 (s), 5.03 (s), 4.58 (m), 4.31 (dd), 3.94 (s), 3.93 (s), 3.92 (s), 3.87 (s), 3.86 (s), 3.47 (br d), 3.27 (dq), 3.13 (dt), 3.07 (dt), 2.87-2.61 (m), 2.34 (br d), 2.26 (br d), 2.18-1.18 (m).

Example 9

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2(S)-carboxylic acid 2-((5-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl) ester (Compound 9):

Compound 9 was prepared as described in Examples 1-2 and 5-7 utilizing 5-hydroxy-1-tetralone in place of 7-hydroxy-1-tetralone to provide Compound 9 as a mixture of diastereomers. ^{1}H NMR as a mixture of diastereomers and rotomers (500 MHz, $CDCl_3$) δ 8.64 (m), 7.39 (m), 7.27 (s), 7.20 (d), 7.17 (q), 6.98 (d), 6.92 (d), 6.80 (t), 6.73 (dd), 6.40 (d), 6.10 (q), 5.99 (t), 5.95 (t), 5.40 (m), 5.12 (m), 5.12 (s), 5.08 (d), 4.60 (m), 4.35 (m), 3.96 (s), 3.85 (s), 3.94 (s), 3.90 (s), 3.89 (s), 3.50 (br d), 3.30 (dq), 3.19-3.08 (m), 3.0-2.86 (m), 2.74-2.58 (m), 2.38 (m), 2.30 (m), 2.10-1.50 (m), 1.45-1.25 (m).

Example 10

1-Amino-7-(pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 10):

To a solution of Compound 1 (1.71 g, 6.75 mmol) and methoxyamine hydrochloride (845 mg, 10.12 mmol) in abs. ethanol (20 mL) was added powdered potassium carbonate (2.25 g, 16.88 mmol) and the reaction heated to reflux. After 2 hr, the reaction was cooled and concentrated in

(Compound 23):

To a solution of Compound 22 (5.73 g, 24.07 mmol) and 85% phosphoric acid (2.36 g, 24.07 mmol) in acetonitirle (50 mL) at 50°C was added trifluoroacetic anhydride (3.5 mL, 25 mmol). After 15 min, the reaction was cooled, diluted with ethyl acetate and washed with water, 10% sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 5% ethyl acetate:hexanes) provided 3.54 g of Compound 23.

Example 24

6-Methyl-5,7-dipropoxy-1,2,3,4-tetrahydronaphthalen-1-one (Compound 24):

To a solution of Compound 23 (3.54 g, 16.1 mmol) in toulene (50 mL was added aluminum chloride (10.7 g, 80.5 mmol) in portions. Once the addition was complete, the mixture was heated to reflux, stirred for 30 min and cooled to 0°C. The reaction was quenched by the addition of 1 N hydrochloric acid and the product extract with ethyl acetate (2x). The extracts were combined, washed with water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue was passed through a plug of silica gel (elution with 20% ethyl acetate:hexanes) to provide 2.78 g of diol. This material was dissolved in 2-butanone (25 mL), treated with 1-bromopropane (6.6 mL, 72.6 mmol) and powdered potassium carbonate (9.68 g, 72.6 mmol) and heated to reflux. After 12 hr the reaction was cooled, diluted with water and extracted with ethyl acetate (2x). The extracts were combined, washed with water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on

(Compound 23):

To a solution of Compound 22 (5.73 g, 24.07 mmol) and 85% phosphoric acid (2.36 g, 24.07 mmol) in acetonitirle (50 mL) at 50°C was added trifluoroacetic anhydride (3.5 mL, 25 mmol). After 15 min, the reaction was cooled, diluted with ethyl acetate and washed with water, 10% sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 5% ethyl acetate:hexanes) provided 3.54 g of Compound 23.

Example 24

6-Methyl-5,7-dipropoxy-1,2,3,4-tetrahydronaphthalen-1-one (Compound 24):

To a solution of Compound 23 (3.54 g, 16.1 mmol) in toulene (50 mL was added aluminum chloride (10.7 g, 80.5 mmol) in portions. Once the addition was complete, the mixture was heated to reflux, stirred for 30 min and cooled to 0°C. The reaction was quenched by the addition of 1 N hydrochloric acid and the product extract with ethyl acetate (2x). The extracts were combined, washed with water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue was passed through a plug of silica gel (elution with 20% ethyl acetate:hexanes) to provide 2.78 g of diol. This material was dissolved in 2-butanone (25 mL), treated with 1-bromopropane (6.6 mL, 72.6 mmol) and powdered potassium carbonate (9.68 g, 72.6 mmol) and heated to reflux. After 12 hr the reaction was cooled, diluted with water and extracted with ethyl acetate (2x). The extracts were combined, washed with water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on

acetone:hexanes) provided Compound 11A. Further elution provided Compound 11B.

Compound 11A: ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.57 (m), 7.36(d), 7.34 (s), 7.30 (d), 7.13 (s), 7.02 (t), 6.97 (d), 6.82 (dd), 6.79 (dd), 6.73 (d), 6.11 (d), 5.21 (m), 5.18-5.08 (m), 5.02 (s), 4.66 (br d), 4.18 (d), 3.92 (s), 3.87 (s), 3.81 (s), 3.60 (br d), 3.32 (dt), 2.81-2.64 (m), 2.40 (br d), 2.26 (m), 2.11-2.01 (m), 1.84-1.65 (m), 1.51-1.42 (m).

Compound 11B: ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.58 (m), 8.48 (m), 7.34 (s), 7.33 (m), 7.29 (m), 7.21 (d), 7.17 (s), 7.02 (t), 6.86 (d), 6.86-6.76 (m), 6.01 (d), 5.19-5.10 (m), 5.02 (m), 4.99 (q), 4.58 (br d), 4.18 (d), 3.93 (s), 3.89 (s), 3.86 (s), 3.48 (br d), 3.41 (dt), 2.80-2.62 (m), 2.41 (br d), 2.21 (br d), 2.12-2.00 (m), 1.88-1.40 (m).

Example 12

N-Benzyl-1-amino-7-(pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 12):

A solution of Compound 1 (820 mg, 3.24 mmol) and benzyl amine (354 µL, 3.24 mmol) in benzene (10 mL) was heated to reflux under azeotropic conditions. After the calculated amount of water was collected, the reaction was cooled and concentrated in vacuo. The residue was taken-up into ethanol (5 mL) and added to a slurry of sodium borohydride (246 mg, 6.48 mmol) in ethanol (15 mL). The reaction was heated to 80°C, stirred for 30 min, cooled and concentrated in vacuo. The residue was diluted with ethyl acetate followed by the slow addition of 1 N hydrochloric acid. The layers were separated. The aqueous phase was adjusted to pH 7 with 2 N sodium

acetone:hexanes) provided Compound 11A. Further elution provided Compound 11B.

Compound 11A: ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.57 (m), 7.36(d), 7.34 (s), 7.30 (d), 7.13 (s), 7.02 (t), 6.97 (d), 6.82 (dd), 6.79 (dd), 6.73 (d), 6.11 (d), 5.21 (m), 5.18-5.08 (m), 5.02 (s), 4.66 (br d), 4.18 (d), 3.92 (s), 3.87 (s), 3.81 (s), 3.60 (br d), 3.32 (dt), 2.81-2.64 (m), 2.40 (br d), 2.26 (m), 2.11-2.01 (m), 1.84-1.65 (m), 1.51-1.42 (m).

Compound 11B: ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.58 (m), 8.48 (m), 7.34 (s), 7.33 (m), 7.29 (m), 7.21 (d), 7.17 (s), 7.02 (t), 6.86 (d), 6.86-6.76 (m), 6.01 (d), 5.19-5.10 (m), 5.02 (m), 4.99 (q), 4.58 (br d), 4.18 (d), 3.93 (s), 3.89 (s), 3.86 (s), 3.48 (br d), 3.41 (dt), 2.80-2.62 (m), 2.41 (br d), 2.21 (br d), 2.12-2.00 (m), 1.88-1.40 (m).

Example 12

N-Benzyl-1-amino-7-(pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalene (Compound 12):

A solution of Compound 1 (820 mg, 3.24 mmol) and benzyl amine (354 µL, 3.24 mmol) in benzene (10 mL) was heated to reflux under azeotropic conditions. After the calculated amount of water was collected, the reaction was cooled and concentrated in vacuo. The residue was taken-up into ethanol (5 mL) and added to a slurry of sodium borohydride (246 mg, 6.48 mmol) in ethanol (15 mL). The reaction was heated to 80°C, stirred for 30 min, cooled and concentrated in vacuo. The residue was diluted with ethyl acetate followed by the slow addition of 1 N hydrochloric acid. The layers were separated. The aqueous phase was adjusted to pH 7 with 2 N sodium

1,2,3,4-tetrahydronaphthalen-1-ol (Compound 28):

To a solution of Compound 26 (1.10 g, 2.98 mmol), in abs. methanol (10 mL) was slowly added sodium borohydride (226 mg, 2.98 mmol). After stirring for 1 hr, the reaction was concentrated and the residue partitioned between ethyl acetate and water. The layers were separated and the organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 10% ethyl acetate:hexanes) provided 502 mg of Compound 27. Further elution provided 475 mg of Compound 28.

Example 28

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2(S)-carboxylic acid (6-methyl-5,7-dipropoxy-2(R)-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester and 1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)piperidine-2(S)-carboxylic acid (6-methyl-5,7-dipropoxy-2(S)-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 29A and 29B):

Compounds 29A and 29B were prepared as described in Examples 5-7, but replacing Compound 2 with Compound 27 to provide a diastereomeric mixture. Chromatography of the mixture on silica gel (elution 10% acetone:hexanes) provided Compound 29A. Further elution provided Compound 29B.

Compound 29A: ^{1}H NMR as a mixture of rotomers (500 MHZ, $CDCl_3$) δ 8.54-8.43 (m), 7.60 (d), 7.41 (s), 7.31 (s), 7.30-7.28 (m), 6.61 (s), 6.57 (s), 5.97 (d), 5.93 (d), 5.40 (d), 4.63 (br d), 4.43 (d), 3.98 (s), 3.97 -3.68 (m), 3.93 (s), 3.89 (s), 3.50 (br d), 3.32 (dt), 3.22

1,2,3,4-tetrahydronaphthalen-1-ol (Compound 28):

To a solution of Compound 26 (1.10 g, 2.98 mmol), in abs. methanol (10 mL) was slowly added sodium borohydride (226 mg, 2.98 mmol). After stirring for 1 hr, the reaction was concentrated and the residue partitioned between ethyl acetate and water. The layers were separated and the organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. Chromatography of the residue on silica gel (elution with 10% ethyl acetate:hexanes) provided 502 mg of Compound 27. Further elution provided 475 mg of Compound 28.

Example 28

1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2(S)-carboxylic acid (6-methyl-5,7-dipropoxy-2(R)-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester and 1-(2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)piperidine-2(S)-carboxylic acid (6-methyl-5,7-dipropoxy-2(S)-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 29A and 29B):

Compounds 29A and 29B were prepared as described in Examples 5-7, but replacing Compound 2 with Compound 27 to provide a diastereomeric mixture. Chromatography of the mixture on silica gel (elution 10% acetone:hexanes) provided Compound 29A. Further elution provided Compound 29B.

Compound 29A: ^{1}H NMR as a mixture of rotomers (500 MHZ, $CDCl_3$) δ 8.54-8.43 (m), 7.60 (d), 7.41 (s), 7.31 (s), 7.30-7.28 (m), 6.61 (s), 6.57 (s), 5.97 (d), 5.93 (d), 5.40 (d), 4.63 (br d), 4.43 (d), 3.98 (s), 3.97 -3.68 (m), 3.93 (s), 3.89 (s), 3.50 (br d), 3.32 (dt), 3.22

3-Benzyl-2(S)-((2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 19):

Compound 19 was prepared according to Examples 5-7, but replacing (S)-Alloc-pipecolic acid with (S)-Alloc-phenylalanine and utilizing Compound 2(R). ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.57 (dd), 7.66(s), 7.52 (d), 7.32-7.23 (m), 7.19 (d), 7.05 (d), 6.87 (m), 6.86 (s), 6.00 (t), 5.03 (q), 4.88 (q), 3.94 (s), 3.88 (s), 3.20 (dq), 2.78 (dt), 2.69-2.63 (m), 1.97-1.73 (m).

Example 20

3-Benzyl-2(S)-(methyl-(2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 20):

Compound 20 was prepared according to Examples 5-7, but replacing (S)-Alloc-pipecolic acid with (S)-Alloc-N-methyl-phenylalanine and utilizing Compound 2(R). ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.55 (d), 8.52 (d), 7.34 (s), 7.31-7.19 (m), 7.12 (m), 7.06-6.99 (m), 6.94-6.82 (m), 6.06 (t), 5.94 (t), 5.05 (q), 4.99 (q), 4.56 (q), 3.90 (s), 3.91 (s), 3.82 (s), 3.75 (s), 3.37 (dd), 3.28 (dd), 3.16 (dd), 3.08 (s), 2.99 (dd), 2.82-2.62 (m), 2.76 (s), 2.05-1.74 (m).

Example 21

3-Benzyl-2(S)-(methyl-(2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester (Compound 21):

3-Benzyl-2(S)-((2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 19):

Compound 19 was prepared according to Examples 5-7, but replacing (S)-Alloc-pipecolic acid with (S)-Alloc-phenylalanine and utilizing Compound 2(R). ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.57 (dd), 7.66(s), 7.52 (d), 7.32-7.23 (m), 7.19 (d), 7.05 (d), 6.87 (m), 6.86 (s), 6.00 (t), 5.03 (q), 4.88 (q), 3.94 (s), 3.88 (s), 3.20 (dq), 2.78 (dt), 2.69-2.63 (m), 1.97-1.73 (m).

Example 20

3-Benzyl-2(S)-(methyl-(2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(R)-yl) ester (Compound 20):

Compound 20 was prepared according to Examples 5-7, but replacing (S)-Alloc-pipecolic acid with (S)-Alloc-N-methyl-phenylalanine and utilizing Compound 2(R). ^{1}H NMR as a mixture of rotomers (500 MHz, $CDCl_3$) δ 8.55 (d), 8.52 (d), 7.34 (s), 7.31-7.19 (m), 7.12 (m), 7.06-6.99 (m), 6.94-6.82 (m), 6.06 (t), 5.94 (t), 5.05 (q), 4.99 (q), 4.56 (q), 3.90 (s), 3.91 (s), 3.82 (s), 3.75 (s), 3.37 (dd), 3.28 (dd), 3.16 (dd), 3.08 (s), 2.99 (dd), 2.82-2.62 (m), 2.76 (s), 2.05-1.74 (m).

Example 21

3-Benzyl-2(S)-(methyl-(2-oxo-2-(3,4,5-trimethoxyphenyl)-acetyl)amino)propanoic acid ((7-pyridin-4-ylmethoxy)-1,2,3,4-tetrahydronaphthalen-1(S)-yl) ester (Compound 21):

20	525	175	75	<50	3.0	7.0	10.5
21	650	120	85	75	6.4	6.8	8.7
28A	350	250	190	<50	1.4	1.8	>7.0
29B	350	200	85	<50	1.8	3.7	>7.0
30A	350	250	150	<50	1.4	2.3	>7.0
30B	350	250	175	<50	1.4	2.0	>7.0

EXAMPLE 32

Inhibition of MRP-Mediated MDR

In order to demonstrate that the compounds of this invention are effective in reversing MPR-mediated MDR, in addition to P-glycoprotein-mediated MDR, we assayed inhibition in a non-P-glycoprotein expressing cell line.

We plated HL60/ADR cells in 96 well microtiter plates (4×10^4 cells/well). The cells were then exposed to various concentrations of doxorubicin (50 nM to 10 µM) in the presence or absence of various compounds of this invention at various concentrations (0.5 - 10 µM). After culturing the cells for 3 days, their viability was quantitated using the XTT dye method to assess mitochondrial function. Results were expressed as a ratio of the IC_{50} for doxorubicin alone to the IC_{50} for doxorubicin plus MDR inhibitor. IC_{50} values are expressed in nM. In all assays the intrinsic antiproliferative or cytotoxicity activity of the MDR inhibitors was also determined for HL60/ADR cells. The results of this assay are set forth in Table 3 below:

Table 3: Reversal Of MRP-mediated MDR in HL60/ADR Cells

20	525	175	75	<50	3.0	7.0	10.5
21	650	120	85	75	5.4	6.8	8.7
29A	350	250	190	<50	1.4	1.8	>7.0
29B	350	200	85	<50	1.8	3.7	>7.0
30A	350	250	150	<50	1.4	2.3	>7.0
30B	350	250	175	<50	1.4	2.0	>7.0

EXAMPLE 32

Inhibition of MRP-Mediated MDR

In order to demonstrate that the compounds of this invention are effective in reversing MPR-mediated MDR, in addition to P-glycoprotein-mediated MDR, we assayed inhibition in a non-P-glycoprotein expressing cell line.

We plated HL60/ADR cells in 96 well microtiter plates (4 x 10^4 cells/well). The cells were then exposed to various concentrations of doxorubicin (50 nM to 10 µM) in the presence or absence of various compounds of this invention at various concentrations (0.5 - 10 µM). After culturing the cells for 3 days, their viability was quantitated using the XTT dye method to assess mitochondrial function. Results were expressed as a ratio of the IC_{50} for doxorubicin alone to the IC_{50} for doxorubicin plus MDR inhibitor. IC_{50} values are expressed in nM. In all assays the intrinsic antiproliferative or cytotoxicity activity of the MDR inhibitors was also determined for HL60/ADR cells. The results of this assay are set forth in Table 3 below:

Table 3: Reversal Of MRP-mediated MDR in HL60/ADR Cells

(t), 1.04 (t), 0.99 (t).

Compound 30B: ^{1}H NMR as a mixture of rotomers (500 MHZ, $CDCl_3$) δ 8.49 (m), 8.43 (d), 8.32(d), 7.57 (m), 7.36 (s), 7.35 (s), 7.30-7.25 (m), 7.18 (s), 6.63 (s), 6.48 (s), 6.35 (s), 6.02 (d), 5.87 (d), 5.77 (d), 5.38 (m), 4.66 (br d), 4.44 (d), 3.98-3.67 (m), 3.52 (br d), 3.44 (br d), 3.33 (dt), 3.26 (dt), 3.14 (dt), 3.01 (br d), 2.88-2.49 (m), 2.32 (m), 2.17 (s), 2.16 (s), 2.12 (s), 2.01 (m), 1.87-1.72 (m), 1.68-1.53 (m), 1.09 (t), 1.04(t), 1.02 (t), 0.98 (t).

Example 30

MDR Sensitization assays

To assay the ability of the compounds according to this invention to increase the antiproliferative activity of a drug, cell lines which are known to be resistant to a particular drug may be used. These cell lines include, but are not limited to, the L1210, P388D, CHO and MCF7 cell lines. Alternatively, resistant cell lines may be developed. The cell line is exposed to the drug to which it is resistant, or to the test compound; cell viability is then measured and compared to the viability of cells which are exposed to the drug in the presence of the test compound.

We have carried out assays using L1210 mouse leukemia cells transformed with the pHaMDR1/A retrovirus carrying a MDR1 cDNA, as described by Pastan et al., Proc. Natl. Acad. Sci. USA, 85, pp. 4486-4490 (1988). The resistant line, labeled L1210VMDRC.06, was obtained from Dr. M. M. Gottesman of the National Cancer Institute. These drug-resistant transfectants had been selected by culturing cells in 0.06 mg/ml colchicine.

Multi-drug resistance assays were conducted by plating cells (2×10^3, 1×10^4, or 5×10^4 cells/well) in 96 well microtiter plates and exposing them to a

(t), 1.04 (t), 0.99 (t).

Compound 30B: ^{1}H NMR as a mixture of rotomers (500 MHZ, $CDCl_3$) δ 8.49 (m), 8.43 (d), 8.32(d), 7.57 (m), 7.36 (s), 7.35 (s), 7.30-7.25 (m), 7.18 (s), 6.63 (s), 6.48 (s), 6.35 (s), 6.02 (d), 5.87 (d), 5.77 (d), 5.38 (m), 4.66 (br d), 4.44 (d), 3.98-3.67 (m), 3.52 (br d), 3.44 (br d), 3.33 (dt), 3.26 (dt), 3.14 (dt), 3.01 (br d), 2.88-2.49 (m), 2.32 (m), 2.17 (s), 2.16 (s), 2.12 (s), 2.01 (m), 1.87-1.72 (m), 1.68-1.53 (m), 1.09 (t), 1.04(t), 1.02 (t), 0.98 (t).

Example 30

MDR Sensitization assays

To assay the ability of the compounds according to this invention to increase the antiproliferative activity of a drug, cell lines which are known to be resistant to a particular drug may be used. These cell lines include, but are not limited to, the L1210, P388D, CHO and MCF7 cell lines. Alternatively, resistant cell lines may be developed. The cell line is exposed to the drug to which it is resistant, or to the test compound; cell viability is then measured and compared to the viability of cells which are exposed to the drug in the presence of the test compound.

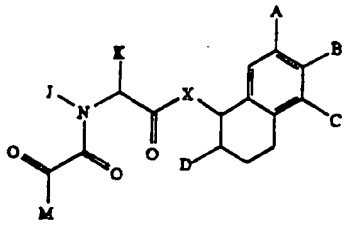
We have carried out assays using L1210 mouse leukemia cells transformed with the pHaMDR1/A retrovirus carrying a MDR1 cDNA, as described by Pastan et al., Proc. Natl. Acad. Sci. USA, 85, pp. 4486-4490 (1988). The resistant line, labeled L1210VMDRC.06, was obtained from Dr. M. M. Gottesman of the National Cancer Institute. These drug-resistant transfectants had been selected by culturing cells in 0.06 mg/ml colchicine.

Multi-drug resistance assays were conducted by plating cells (2×10^3, 1×10^4, or 5×10^4 cells/well) in 96 well microtiter plates and exposing them to a

CLAIMS

I claim:

1. A compound represented by formula (I):



Formula (I)

and pharmaceutically acceptable salts thereof, wherein:

A, B and C are independently selected from hydrogen, halogen, (C1-C6)-straight or branched alkyl, O-(C1-C6)-straight or branched alkyl, $(CH_2)_n$-Ar or $Y(CH_2)_n$-Ar; wherein

Y is O, S or NR_1; wherein

R_1 is (C1-C6)-straight or branched alkyl and hydrogen;

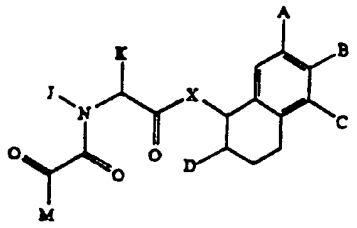
n is an integer from 0 to 4; and

Ar is a carbocyclic aromatic group selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl and anthracenyl; or a heterocyclic aromatic group selected from the group consisting of 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyl, isotriazolyl, 1,2,3-oxadiazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indolizinyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, benzo[b]furanyl,

CLAIMS

I claim:

1. A compound represented by formula (I):



Formula (I)

and pharmaceutically acceptable salts thereof, wherein:

A, B and C are independently selected from hydrogen, halogen, (C1-C6)-straight or branched alkyl, O-(C1-C6)-straight or branched alkyl, $(CH_2)_n$-Ar or $Y(CH_2)_n$-Ar; wherein

Y is O, S or NR_1; wherein

R_1 is (C1-C6)-straight or branched alkyl and hydrogen;

n is an integer from 0 to 4; and

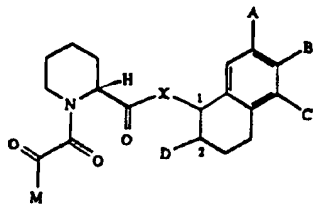
Ar is a carbocyclic aromatic group selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl and anthracenyl; or a heterocyclic aromatic group selected from the group consisting of 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indolizinyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, benzo[b]furanyl,

membered benzo-fused ring;

M is (C1-C6)-straight or branched alkyl or Ar; and

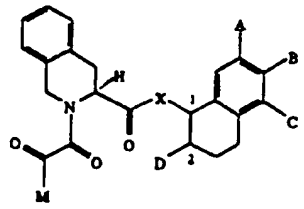
the stereochemistry at carbon 1 and carbon 2 is independently selected from R or S.

2. The compound according to claim 1 represented by formula (II):



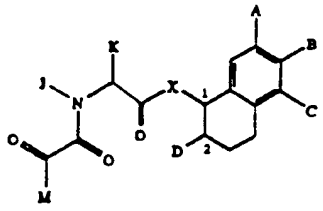
Formula (II)

3. The compound according to claim 1 represented by formula (III):



Formula (III)

4. The compound according to claim 1 represented by formula (IV):



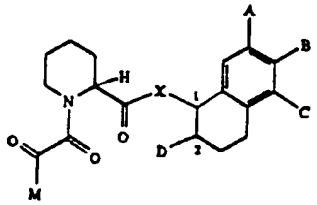
Formula (IV);

membered benzo-fused ring;

M is (C1-C6)-straight or branched alkyl or Ar; and

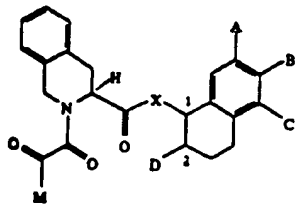
the stereochemistry at carbon 1 and carbon 2 is independently selected from R or S.

2. The compound according to claim 1 represented by formula (II):



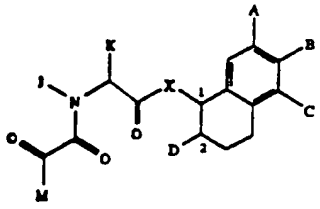
Formula (II)

3. The compound according to claim 1 represented by formula (III):



Formula (III)

4. The compound according to claim 1 represented by formula (IV):



Formula (IV);

any one of claims 1 to 5 or 9 to 12 effective to reduce multi-drug resistance; and

b. A physiologically acceptable adjuvant, carrier or vehicle.

13. The pharmaceutical composition according to claim 12, further comprising a chemotherapeutic agent.

14. The pharmaceutical composition according to claim 12 further comprising a chemosensitizer.

15. A method for treating or preventing multi-drug resistance, comprising the step of administering to said patient a composition according to any one of claims 12 to 14.

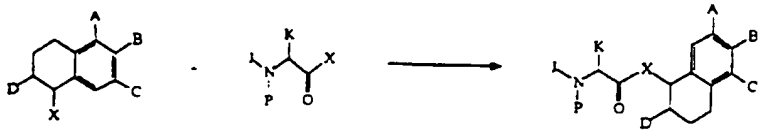
16. The method according to claim 15, wherein said composition is administered orally.

17. The method according to claim 16, wherein said multi-drug resistance is P-glycoprotein-mediated.

18. The method according to claim 17, wherein said multi-drug resistance is MRP-mediated.

19. A process for preparing a compound of formula (I), comprising the steps of:

a. coupling an alcohol or amine of formula (V) with an amino acid of formula (VI) to give the corresponding ester or amide of formula (VII),



(V) (VI) (VII)

any one of claims 1 to 5 or 9 to 12 effective to reduce multi-drug resistance; and

b. A physiologically acceptable adjuvant, carrier or vehicle.

13. The pharmaceutical composition according to claim 12, further comprising a chemotherapeutic agent.

14. The pharmaceutical composition according to claim 12 further comprising a chemosensitizer.

15. A method for treating or preventing multi-drug resistance, comprising the step of administering to said patient a composition according to any one of claims 12 to 14.

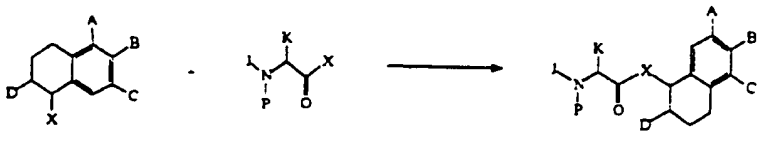
16. The method according to claim 15, wherein said composition is administered orally.

17. The method according to claim 16, wherein said multi-drug resistance is P-glycoprotein-mediated.

18. The method according to claim 17, wherein said multi-drug resistance is MRP-mediated.

19. A process for preparing a compound of formula (I), comprising the steps of:

a. coupling an alcohol or amine of formula (V) with an amino acid of formula (VI) to give the corresponding ester or amide of formula (VII),



(V) (VI) (VII)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 96/07094

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D401/12 C07D213/30 A61K31/44 A61K31/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 634 401 (AMERICAN CYANAMID CO) 18 January 1995 see claims; example 119 ---	1-19
A	WO,A,94 07858 (VERTEX PHARMA) 14 April 1994 cited in the application see the whole document ---	1-19
A	BUDAVARI, S. (EDITOR): "The Merck Index, 11th edition" 1989 , MERCK & CO., INC. , RAHWAY, N.J., USA XP002011527 cited in the application see Appendix A5: FK-506 --- -/--	1-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 1996	02.09.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bosma, P

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PCT/US 96/07094

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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 634 401 (AMERICAN CYANAMID CO) 18 January 1995 see claims; example 119 ---	1-19
A	WO,A,94 07858 (VERTEX PHARMA) 14 April 1994 cited in the application see the whole document ---	1-19
A	BUDAVARI, S. (EDITOR): "The Merck Index, 11th edition" 1989 , MERCK & CO., INC. , RAHWAY, N.J., USA XP002011527 cited in the application see Appendix A5: FK-506 --- -/--	1-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 1996	02.09.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bosma, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07094

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 15-18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07094

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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